Technical Bulletin

Randomized Non-inferiority Trial Comparing Two Commercial Intramammary Antibiotics for the Treatment of Non-severe Clinical Mastitis in Dairy Cows

Vasquez AK, Nydam DV, Capel MB, et al. *J. Dairy Sci.* 2016;90(10):8267-8281.

Key Points:

- No significant difference in overall bacteriologic cure risk between treatments.
- No significant difference in clinical cure risk, milk production, linear score, mastitis recurrence or culling between treatments.
- Cows treated with PolyMast® (hetacillin potassium) spent significantly fewer days in the hospital pen.
- Study results can be used as the basis to develop farm-specific treatment protocols for clinical mastitis.

Introduction

Clinical mastitis is an important disease of dairy cattle, requiring dairy producers to make treatment decisions upon observation of symptoms to minimize production and milk quality losses. Several treatment options are available to dairy producers; however, most efficacy information available evaluates a treatment against a negative control. Comparative studies, evaluating one registered intramammary (IMM) treatment against another, would be valuable in making information-based treatment decisions.

Both intramammary treatments evaluated here are effective against one or more Gram-positive organisms, as well as Gram-negative *E. coli*. Although both treatments have a milk withdrawal time of 72 hours, average length of treatment varies. For POLYMAST, treatments may be repeated three times at 24-hour intervals, while the label for Spectramast® LC (ceftiofur hydrochloride) states it has the flexibility of two to eight treatments at 24-hour intervals. Average protocol length for use of ceftiofur is between four and five days[1]. Total time out of the tank (intra-treatment interval and milk withdrawal) for POLYMAST-treated cows would be 120 hours, while for a five-day SPECTRAMAST LC protocol, cows would be out of the tank for a total of 168 hours. If non-inferiority could be established, there would be a significant economic advantage for the shorter-duration protocol with POLYMAST, as well as promotion of judicious antimicrobial usage, over SPECTRAMAST LC.

Study Objective

To compare the treatment efficacy of a three-day, 24-hour interval treatment with PolyMast® (hetacillin potassium) to a five-day, 24-hour interval treatment with Spectramast® LC (ceftiofur hydrochloride).

Materials and Methods

The study design was a non-inferiority study. To disprove the null hypothesis, POLYMAST must not be inferior to SPECTRAMAST LC by more than 15%. The margin of 15% was chosen because of the economic advantage of a shorter-duration protocol with POLYMAST over an extended-therapy protocol with SPECTRAMAST LC.

Farms with at least 500 cows, and participating in monthly Dairy Herd Improvement Association (DHIA) testing, including somatic cell counting (SCC), were eligible. A known presence of streptococcal mastitis was also required to ensure appropriate representation of Gram-positive and Gram-negative infections.

To be included in the study, cows must have had a parity fewer than six, had fewer than 300 days in milk (DIM), had no clinical mastitis or antimicrobial treatment for any reason within the prior 30 days, and had clinical mastitis in only one quarter that was classified as mild or moderate. Cows with severity scores >2 were not eligible for enrollment.

After mild or moderate clinical mastitis was identified, farmworkers obtained a milk sample for culture, recorded a clinical score, and treated the animal with the test treatment as randomly assigned upon opening of a numbered study envelope.

Clinical scores were recorded on the day of enrollment, and then daily for four additional days. Milk samples were taken for follow-up culture, and clinical scores recorded approximately 14 days (+/-4) and 21 (+/-5) days after onset of treatment.

Milk culture and pathogen identification techniques were in accordance with National Mastitis Council guidelines.

Outcomes

Bacteriologic cure: A quarter was defined as a bacteriologic cure when the pathogen present in the enrollment sample was not present in either post-treatment sample. Cows that initially cultured with no growth or contamination were not included in this analysis.

Pathogen cure: Pathogen cure was included in the study to facilitate the inclusion of “no-growth” samples and the risk of remaining pathogen-free. If both follow-up samples contained no growth, the cow was defined as a pathogen cure.

Clinical cure: A quarter was defined as a clinical cure when, within the first five days of observation, the clinical score became “0.”

Other outcomes: Extended clinical cure, number of clinical days, post-treatment milk production and linear score, survival of the cow in the herd, occurrence of another mastitis event in the same quarter and hospital days.

Statistical Analysis

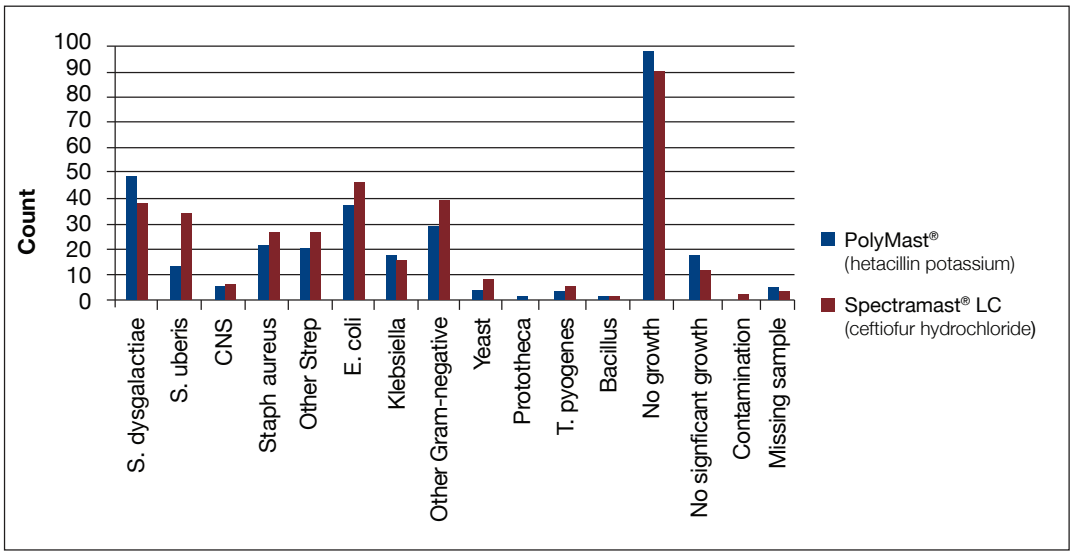
Non-inferiority analysis of binary outcomes (bacteriologic cure, pathogen cure, clinical cure by day 4, survival to day 30, and survival to day 60) was completed using PROC FREQ in SAS®. Statistical analysis of the primary and secondary outcomes was performed using regression models, taking into account parity, DIM, hospital days, previous milk yield, linear score (LS) and clinical mastitis etiology.

Results

Six New York farms met eligibility requirements and were included in the study, contributing a total of 627 cases. Thirty-one cases did not remain in the study, due to secondary treatment of the cow or quarter within the first seven days, non-survival of cow or quarter, or input errors. No difference in age, days in milk or affected quarter existed between the treatment groups.

Culture results (n = 588) from the initial milk samples taken at the time of first treatment are shown in Figure 1. Thirty-seven percent of samples were caused by Gram-positive organisms, 28% were caused by Gram-negative organisms, 28% of samples were no-growth, and 7% of samples were classified as "other" (contaminated, yeast, Prototheca, etc.).

Figure 1. Intramammary Infection Status at Time of First Treatment



Cases were only eligible for bacteriologic cure if the quarter was culture-positive at study entry: Three hundred fifty-three cases (164 in the POLYMAST group and 189 in the SPECTRAMAST LC group) had complete data and were analyzed. Overall bacteriologic cure was 71%: 68% for POLYMAST and 73% for SPECTRAMAST LC. The odds ratio for cure was 1.3 times higher for SPECTRAMAST LC, but was not significantly different than 1 (p = 0.32). Results were also analyzed by Gram-positive or Gram-negative status. For Gram-positive cases, 63% of the POLYMAST cows cured and SPECTRAMAST LC cure risk was 70%. For cows infected with Gram-negative bacteria, 79% of POLYMAST cows cured, compared with 82% of the SPECTRAMAST LC cows. Additionally, there was no difference between treatments for pathogen cure (p = 0.57).

Clinical cure was observed for 472 cows (PolyMast® (hetacillin potassium) = 268, Spectramast® LC (ceftiofur hydrochloride) = 204). Sixty-four percent of cows experienced a clinical cure in less than four days. A higher percentage (70%) of POLYMAST cows experienced a clinical cure in less than 4 days than SPECTRAMAST LC cows (59%, $p = 0.007$). There was no difference in extended clinical cure at 14 days and 21 days between the treatment groups. Bacteriologic and clinical cure risk results by organism are displayed in Figures 2 and 3.

For post-event milk yield ($p = 0.54$), post-event linear score ($p = 0.66$), survival to day 30 ($p = 0.73$), survival to day 60 ($p = 0.26$), and mastitis recurrence ($p = 0.73$), there was no difference between treatment groups. The only significant difference was in hospital days: 6.2 days for POLYMAST-treated cows versus 8.0 days for SPECTRAMAST LC–treated cows ($p < 0.001$).

Figure 2. Bacteriologic and Clinical Cure (Gram-Positive)

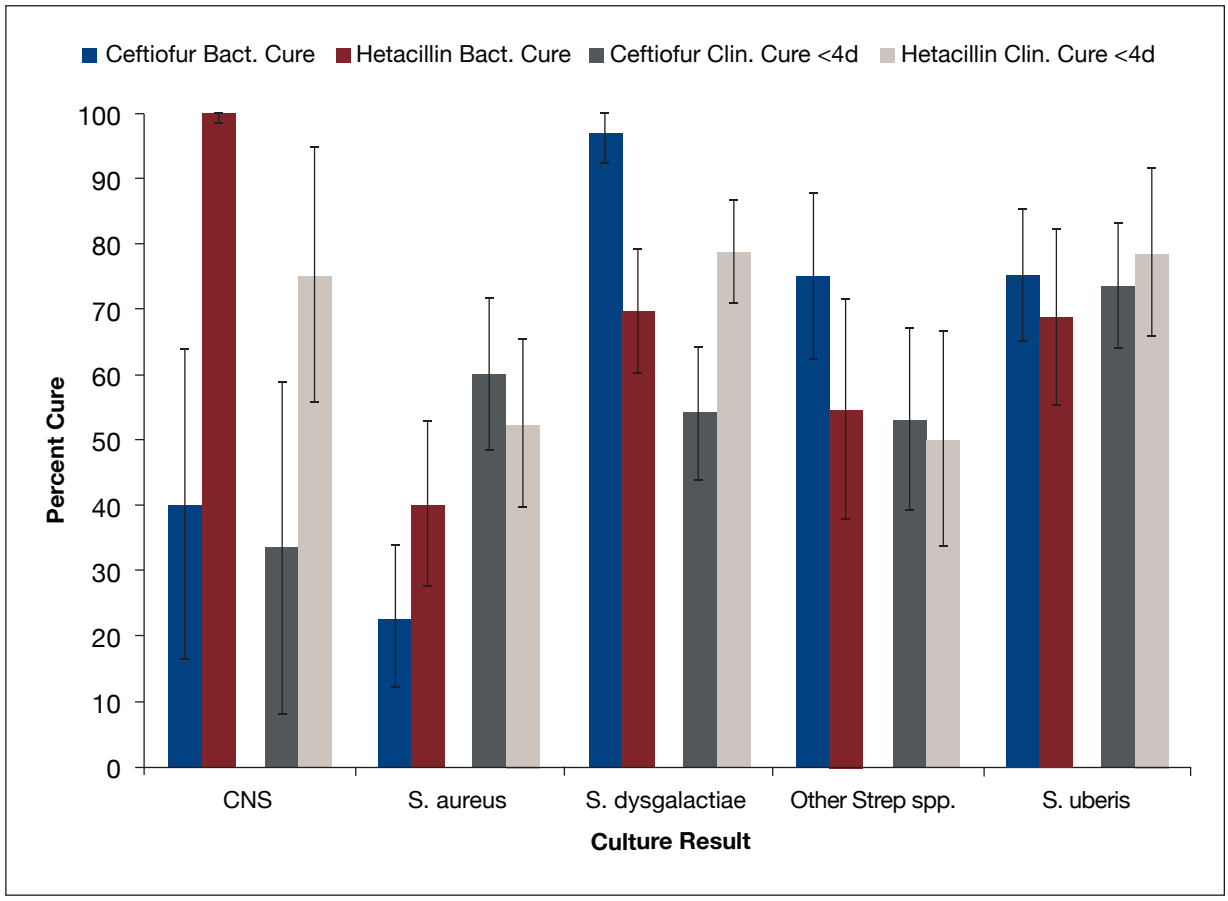
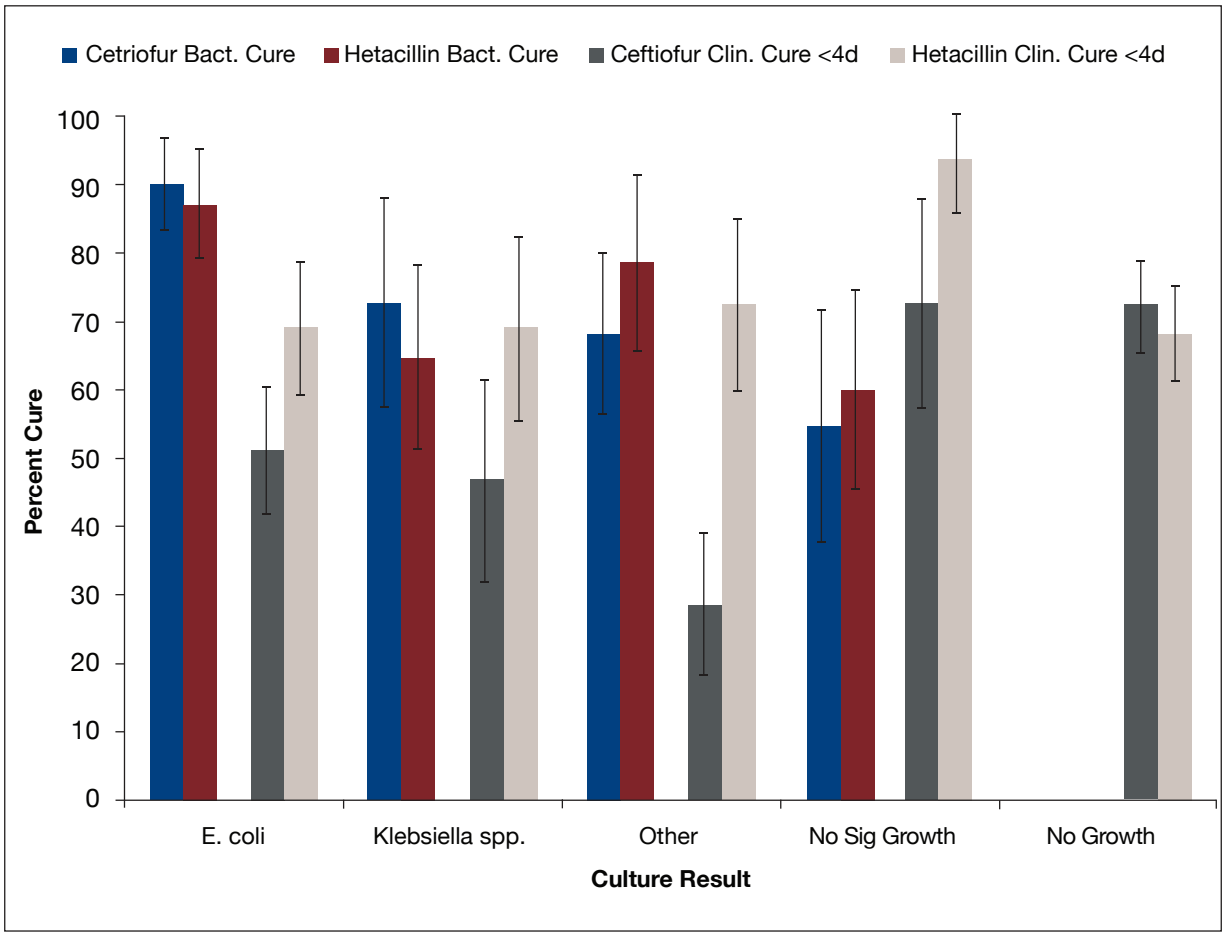


Figure 3. Bacteriologic and Clinical cure (Gram-Negative and No-Growth)



Discussion

This positive-control, randomized trial evaluated the difference in treatment efficacy between PolyMast® (hetacillin potassium) and Spectramast® LC (ceftiofur hydrochloride) for non-severe clinical mastitis. This study found that POLYMAST was not inferior to SPECTRAMAST LC in the primary outcomes of bacteriological, pathogen and clinical cures, and in the secondary outcomes describing survival to 30 and 60 days. When broken down by mastitis etiologies, non-inferiority of POLYMAST to SPECTRAMAST LC was inconclusive, likely due to the small sample size when results were separated by Gram-negative and Gram-positive status. POLYMAST was superior when clinical days are considered.

Conclusion

Using a five-day extended approach for the treatment of all mastitis cases may not be economically justifiable, when cost of the intramammary tubes, labor, milk discard and time in the hospital pen is considered. Since cows treated with PolyMast® (hetacillin potassium) spend less time in the hospital pen, a three-day protocol implementing POLYMAST would result in a 120-hour milk discard versus a 168-hour milk withhold for a five-day protocol with Spectramast® LC (ceftiofur hydrochloride). For herds that do not utilize a culture-based approach for treatment of clinical mastitis, there is economic benefit to choosing a tube with a shorter period of nonsalable milk as the first treatment of choice. Additionally, choosing a semi-synthetic penicillin over a third-generation cephalosporin demonstrates judicious use of antibiotics in mastitis therapy.

Reference

[1] Oliviera and Ruegg. Treatment of clinical mastitis occurring in cows on 51 large dairy herds in Wisconsin. *J Dairy Sci.* 2014;97(9):5426-5436.

NADA 055-054, Approved by FDA

PolyMast®

(hetacillin potassium)

Intramammary Infusion

For lactating cows only

Boehringer Ingelheim

Caution: Federal law restricts this drug to use by or on the order of a licensed veterinarian.

Description: PolyMast is a broad-spectrum agent which provides bactericidal activity against a wide range of common Gram-positive and Gram-negative bacteria. It is derived from 6-aminopenicillanic acid and is chemically related to ampicillin.

Each 10 mL disposable syringe contains hetacillin potassium equivalent to 62.5 mg ampicillin activity in a stable peanut oil gel. This product was manufactured by a non-sterilizing process.

Storage: Do not store above 25°C (77°F). Do not freeze.

Action: Hetacillin provides bactericidal levels of the active antibiotic, ampicillin. *In vitro* studies have demonstrated susceptibility of the following organisms to ampicillin: *Streptococcus agalactiae, Streptococcus dysgalactiae, Staphylococcus aureus* and *Escherichia coli*.

Indications: For the treatment of acute, chronic or subclinical bovine mastitis. PolyMast for intramammary infusion should be used at the first signs of inflammation or at the first indication of any alteration in the milk. Subclinical infections should be treated immediately upon determining, by C.M.T. or other tests, that the leukocyte count is elevated, or that a susceptible pathogen has been cultured from the milk.

PolyMast for intramammary infusion has been shown to be efficacious in the treatment of mastitis in lactating cows caused by susceptible strains of *Streptococcus agalactiae, Streptococcus dysgalactiae, Staphylococcus aureus* and *Escherichia coli.*

Polycillin (ampicillin) Susceptibility Test Discs, 10 mcg, should be used to estimate the *in vitro* susceptibility of bacteria to hetacillin.

Dosage and Administration: Infuse the entire contents of one syringe (10 mL) into each infected quarter. Repeat at 24-hour intervals until a maximum of three treatments has been given.

If definite improvement is not noted within 48 hours after treatment, the causal organism should be further investigated.

Wash the udder and teats thoroughly with warm water containing a suitable dairy antiseptic and dry, preferably using individual paper towels. Carefully scrub the teat end and orifice with 70% alcohol, using a separate swab for each teat.

Allow to dry.

PolyMast is packaged with the Opti-Sert® protective cap.

For Partial Insertion: Twist off upper portion of the OPTI-SERT protective cap to expose 3–4 mm of the syringe tip.

For Full Insertion: Remove protective cap to expose the full length of the syringe tip.

Insert syringe tip into the teat canal and expel the entire contents of one syringe into each infected quarter. Withdraw the syringe and gently massage the quarter to distribute the medication.

Do not infuse contents of the mastitis syringe into the teat canal if the OPTI-SERT protective cap is broken or damaged.

Residue Warnings: 1. Milk that has been taken from animals during treatment and for 72 hours (6 milkings) after the latest treatment must not be used for food.
2. Treated animals must not be slaughtered for food until 10 days after the latest treatment.

Precautions: Because it is a derivative of 6-aminopenicillanic acid, PolyMast has the potential for producing allergic reactions. Such reactions are rare; however, should they occur, treatment should be discontinued and the subject treated with antihistamines, pressor amines, such as epinephrine or corticosteroids.

The drug does not resist destruction by penicillinase and, hence, is not effective against strains of staphylococcus resistant to penicillin G.

How Supplied: PolyMast intramammary infusion is supplied as 10 mL syringes containing 62.5 mg ampicillin activity per syringe.
One display carton contains 12 syringes. One pail contains 144 syringes.

NDC 0010-4722-01 - 10 mL syringe; NDC 0010-4722-02 -12 syringes; NDC 0010-4722-03 -144 syringes.

Made in Italy
Manufactured for:
Boehringer Ingelheim Animal Health USA Inc.
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